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Running title: soft tissue augmentation at implant sites

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CLINICAL RELEVANCE

Scientific rationale for the study: Autogenous subepithelial connective tissue grafts (SCTGs) are the gold standard to augment soft tissue volume at implant sites. In order to reduce the morbidity, research activities have focused on soft tissue substitutes. A recently developed three-dimensionally stable collagen matrix (VCMX) demonstrated favorable biological properties in preclinical studies. This clinical study aimed to confirm the effectiveness and safety of VCMX in comparison to SCTG.

Principal findings: Soft tissue augmentation was a safe procedure increasing the soft tissue thickness up to 1.6 mm without relevant differences between VCMX and SCTG**.**

Practical implications: VCMX might replace SCTGs for the augmentation of soft tissue volume at implant sites in the future.

ABSTRACT

Aim: To test whether or not the use of a collagen matrix (VCMX) results in short-term soft tissue volume increase at implant sites non-inferior to an autogenous subepithelial connective tissue graft (SCTG) and, to evaluate safety and tissue integration of VCMX and SCTG.

Methods: In 20 patients with a volume deficiency at single-tooth implant sites, soft tissue volume augmentation was performed randomly allocating VCMX or SCTG. Soft tissue thickness, patient-reported outcome measures (PROMs), and safety were assessed up to 90 days (FU-90). At FU-90 (abutment connection), tissue samples were obtained for histological analysis. Descriptive analysis was computed for both groups. Nonparametric tests were applied to test non-inferiority for the gain in soft tissue thickness at the occlusal site.

Results: Median soft tissue thickness increased between BL and FU-90 by 1.8mm (Q1:0.5;Q3:2.0) (VCMX) (p=0.018) and 0.5mm (-1.0;2.0) (SCTG) (p=0.0.395) (occlusal) and by 1.0mm (0.5;2.0) (VCMX) (p=0.074) and 1.5mm (-2.0;2.0) (SCTG) (p=0.563) (buccal). Non-inferiority with a non-inferiority margin of 1mm could be demonstrated (p=0.020), the difference of the two group medians (1.3mm) for occlusal sites indicated no relevant, but not significant superiority of VCMX vs. SCTG (primary endpoint). Pain medication consumption and pain perceived were non-significantly higher in group SCTG up to day 3. Median physical pain (OHIP-14) at day 7 was 100% higher for SCTG than for VCMX. The histological analysis revealed well-integrated grafts.

Conclusions: Soft tissue augmentation at implant sites resulted in a similar or higher soft tissue volume increase after 90 days for VCMX vs. SCTG. PROMs did not reveal relevant differences between the two groups.

INTRODUCTION

Immediately following tooth extraction biological processes are initiated, which can lead to bone resorption and result in localized alveolar ridge defects (Van der Weijden et al., 2009, Araujo and Lindhe, 2009). A number of surgical techniques were proposed to correct localized alveolar defects (Prato et al., 2004). Depending on the dimension and the location of the site and depending on the restorative treatment planning the use of bone augmentation (Aghaloo and Moy, 2007, Jensen and Terheyden, 2009, Milinkovic and Cordaro, 2014, Chiapasco et al., 2009) as well as soft tissue augmentation (Eghbali et al., 2014, De Bruyckere et al., 2015, Esposito et al., 2012) or a combination of both procedures (Schneider et al., 2011, Cosyn et al., 2015) have been reported.

According to two recent systematic reviews, autogenous subepithelial connective tissue grafts (SCTGs) are considered to be the gold standard to augment soft tissue volume at implant sites and in partially edentulous patients (Thoma et al., 2009, Thoma et al., 2014a). Limitations and disadvantages, however, are associated with the use of autogenous tissue. These typically include i) the height, length and thickness of the donor tissue vary with the different anatomic dimensions of the palatal vault (Benninger et al., 2012), ii) length and thickness are limited by anatomical factors such as a thick alveolar process, exostosis and the palatine nerves and blood vessels (Kim et al., 2014, Yu et al., 2014, Fu et al., 2011), iii) patients often complain about pain and numbness especially in the donor site for several weeks after the surgery (Zucchelli et al., 2010, Griffin et al., 2006, Cairo et al., 2012, Soileau and Brannon, 2006). In order to reduce the morbidity due to the harvesting procedures and overcome the above-mentioned issues of autogenous grafts, research activities have focused on the development of soft tissue substitutes of various origins and for a number of clinical indications (Zuhr et al., 2014, Vignoletti et al., 2014). For the purpose of soft tissue volume augmentation, a suitable device needs to fulfill two main criteria: i) volume stability over time, ii) favorable biological behavior allowing modeling and remodeling processes. Most soft tissue substitutes brought on the market in recent years fulfilled the latter criteria.

Effective volume increase and volume stability over time has not been shown yet. Recently, a three-dimensionally stable collagen matrix was developed. This matrix demonstrated i) favorable mechanical properties and biological attributes promoting the ingrowth of human fibroblasts (Mathes et al., 2010), ii) favorable tissue integration and promotion of angiogenesis (Thoma et al., 2012b) and iii) non-inferiority compared to the gold standard in terms of two- and three-dimensional volume increase in a preclinical model (Thoma et al., 2010, Thoma et al., 2011). Still, data from in vitro and experimental preclinical studies may be difficult to transfer into the clinic. Therefore, the aim of the present randomized controlled clinical trial was i) to test whether or not the use of a collagen matrix (VCMX) results in a soft tissue volume increase at implant sites non-inferior to a subepithelial connective tissue graft (SCTG), ii) to evaluate safety and tissue integration of VCMX and SCTG.

MATERIALS AND METHODS

Study design

This study was designed as a randomized controlled clinical trial and performed in accordance with the ISO Standard 14155:2011, Clinical Investigation of medical devices for human patients with the appendices VIII and X of the Medical Device Directive 93/42/EEC and with the Declaration of Helsinki, 2004. Upon approval by the local ethics committee (KEK-ZH-Nr 2011-0408), patients in need of soft tissue volume increase at single-tooth implant sites were consecutively recruited, informed and screened for inclusion. The following inclusion criteria were applied:

Inclusion criteria:

1. Implant placement at least 6 weeks and maximum 6 months prior enrolment
2. Necessity of soft tissue augmentation in a single tooth gap
3. 2 teeth adjacent at each side of the defect with a mean BOP of < 30%
4. Basic periodontal examination (BPE <2)
5. 18 years or older
6. Ability to comply with the study-related procedures such as exercising

good oral hygiene and attending all follow-up examinations

7. Ability to fully understand the nature of the proposed surgery and ability to sign the informed consent form

Exclusion criteria:

1. Heavy smoker (> 10 cigarettes per day)
2. Probing depth greater than 4 mm
3. Insulin-dependent diabetes
4. General contraindications for dental and/or surgical treatment
5. History of malignancy, radiotherapy, or chemotherapy for malignancy within the past five years
6. Women of child bearing age, not using a standard accepted method for contraception
7. Pregnancy or breast feeding

8. Previous and concurrent medication affecting mucosal healing in general (e.g. steroids, large doses of anti-inflammatory drugs)

9. Disease affecting connective tissue metabolism (e.g. collagenases)

10. Allergy to collagen

Clinical procedures

Screening

Following inclusion in the study, patients were scheduled for a screening visit. At this time-point a number of clinical measurements were performed including basic periodontal parameters and impression taking of the augmentation site (for details see below and Figures 1 and 2A).

Soft tissue augmentation surgery

At the day of surgery, patients rinsed with 0.2% chlorhexidine solution (Hibitan, Astra-Zeneca) for 60 seconds and were then premedicated with 500mg mefenaminacid (Ponstan 500, Parke-Davis) and 1.5g of amoxicilline (Amoxicilline, Sandoz). Following local anesthesia, sulcular incisions were made around the neighbouring teeth and a straight incision from the lingual/palatal line angle of the distal tooth to the lingual/palatal line angle of the mesial tooth. A full thickness flap was elevated on top of the ridge crest between the two neighboring teeth. At the border between the ridge crest and the buccal aspect, a split thickness flap was prepared by a sharp dissection using a blade (Figure 2B). Thereby, the periosteum was not elevated on the buccal aspect. Subsequently, the split thickness flap on the buccal side was extended to prepare a pouch. The dimension of the buccal pouch exceeded the expected size of the transplant. In addition, periosteal releasing incisions were made on the buccal side to allow for a tension-free wound closure. At this time-point a sealed envelope containing the randomly assigned treatment modality was opened.

Treatment modalities:

- cross-linked volume-stable collagen matrix (VCMX) (test)
- autogenous subepithelial connective tissue graft (SCTG) (control)

In group VCMX, the matrix was shaped to match the desired size in the recipient bed (original dimension: 8mm x 8mm x 5mm). In group SCTG, an autogenous connective tissue graft was harvested using a single incision technique. The VCMX/SCTG was then positioned in the pouch under the elevated buccal flap (Figure 2C). A horizontal single suture was made to immobilize the VCMX/SCTG, connecting it to the lingual flap (Gore Tex 5-0, W.L.Gore & Associates, Inc). One horizontal mattress suture (Gore Tex 5-0, W.L.Gore & Associates, Inc) was placed over the buccal prominence created by the volume gain through the applied VCMX/SCTG in order to stabilize the augmented area and to approach the wound margins. Finally, single interrupted sutures (3-5) closed the wound bed (Figure 2D). In group SCTG, the palatal donor site was closed using a horizontal cross-section suture. The overall surgical time to perform the soft tissue augmentation procedures was calculated in minutes. Patients received prescriptions for analgesic and anti-inflammatory medications for three days (Ponstan®, Parke-Davis) and were instructed to rinse with a 0.2% solution of chlorhexidine (Hibitan, Astra-Zeneca) twice a day for 10 days. Additionally, the patients were instructed to take 2.25 g amoxicilline (Amoxicilline, Sandoz) per day for 7 days. Any temporary removable partial denture was checked and adapted if necessary to avoid trauma to the surgical area.

Follow-up examinations (Suture Removal (SR); FU-30; FU-90)

Sutures were removed 7-10 days after the surgery and teeth were professionally cleaned with a mild abrasive prophylaxis paste (suture removal = SR). In addition, the soft tissue healing was assessed at the target and donor site (if applicable) (Figure 2E). Any kind of dehiscence or swelling was recorded at target and donor site. In addition, patient-reported outcome measures (PROMs) were assessed (see below and Figure 1). At 30 (Figure 2F) and 90 days post surgery (FU-30; FU-90), periodontal parameters, the soft tissue healing and PROMs were recorded. In addition, at FU-90, a minimally invasive abutment connection was performed using a u-shaped incision design (Figure 2G). The cover screw of the implant was removed and a small biopsy (roughly 2x2mm) including soft tissue on top of the implant harvested. The small flap was placed underneath the

buccal pouch and a healing abutment connected to the implant (Figure 2H). Similar to FU-30, PROMs were assessed. The performance of the treatment *per se* was judged subjectively at FU-90 by the clinicians as being successful (gain in volume) or unsuccessful (need for an additional soft tissue grafting procedure).

Outcome measures

Assessment of soft tissue thickness at BL (=prior to surgery), at 30 days, at 90 days

The primary efficacy outcome was the gain in mucosal soft tissue thickness (measured at the occlusal aspect) at day 90 compared to day 0 (baseline value) measured by transmucosal probing.

In order to assess the changes in soft tissue thickness at the target site, an alginate impression was taken at screening and an individualized stent with three standardized openings (occlusal, buccal, apical) fabricated by means of CAD/CAM technology according to a previously published protocol (Figure 3A) (Thoma et al., 2012a). Transmucosal probing for mucosal thickness was performed with an endodontic instrument (RS STER K-File 31/15, Dentsply Maillefer). The stent served as a guide for reproducible measurements of the soft tissue thickness. After placing the individualized stent, the soft tissue thickness was measured by introducing the endodontic instrument in the openings and penetrating the mucosa to the bone (Figure 3B). With an additional measurement the tip of the endodontic instrument stopped on top of the mucosa. The difference between the two measurements represented the soft tissue thickness.

Assessment of periodontal status

Standard clinical and periodontal measurements were performed at BL, at 30 days and at 90 days and included: the basic periodontal examination (BPE) in all sextants, plaque index (PlI) at six sites around the neighboring teeth according to Silness-Löe, the width of keratinized tissue (KT) at the buccal side of the two neighboring teeth, bleeding on

probing (BOP) as present or not at the two neighboring teeth, and periodontal probing depth (PPD), clinical attachment level (CAL) and recession depth (RC) at six sites around the two neighboring teeth.

Patient-reported outcome measures (PROMs)

PROMs were assessed calculating the overall consumption of analgesics (Ponstan 500, Parke-Davis) during the entire study period using self-reported questionnaires handed-out to the patients at each visit and being collected at the follow-up visit. Pain perceived by patients between the follow-up visits was assessed by documenting the intake of analgesics / anti-inflammatory medication (Ponstan 500, Parke-Davis). During the first 7-10 days after surgery, overall pain was daily assessed by the patients using a questionnaire (visual analog scale = VAS), thereafter one of the investigators (MZ) recorded pain at FU-30 and FU-90 using the same VAS. In addition, an oral health impact profile questionnaire (OHIP-G14) was handed out to patients and filled out at SC (screening, baseline=BL), SR and FU-90.

Safety evaluations

Any adverse event and complication as well as wound closure, the presence of swelling and concomitant medication were recorded during the entire study period.

Histological preparation and assessment

Obtained biopsies were fixed, decalcified, dehydrated in alcohol solutions of increasing concentration, cleared in isoparaffin H and embedded in paraffin. Embedded samples were cut at 5 µm using a microtome. One section per block was prepared and stained with Van Gieson-Elastica to study the remaining amount of the VCMX and the newly formed connective tissue (group VCMX), whereas in group SCTG, the overall amount of connective tissue was calculated. All histological sections were evaluated using a microscope for qualitative and semi-quantitative histological analysis. For histomorphometrical analysis, the digitized histological images were analyzed using an

image-processing program. The following parameters were assessed within a defined region of interest in the center of the biopsy: i) connective tissue, ii) remaining VCMX, iii) background. One masked experienced examiner performed all the measurements.

Statistical analysis

Mean, median, standard deviation and the range were used to describe the continuously scaled variables and counts and percentages for categorically scaled variables. Nonparametric statistical methods were applied. The differences of the medians between the treatment groups were evaluated with the Mann-Whitney and within a treatment group with the Wilcoxon signed rank test.

The data were analyzed as intention-to-treat set (ITT: all randomized patients with post-baseline data) as well as per protocol analysis set (PP: ITT without major protocol violations). No relevant differences were found between the results in both analysis sets. The results for the primary objective are therefore presented as PP analysis set (ITT analysis is not generally conservative in non-inferiority trials).

Descriptive statistics and simple comparisons are reported for the ITT analysis set. In order to demonstrate that the two treatment groups do not differ relevantly with respect to the medians of change in soft tissue thickness (Δ_{VCMX} and Δ_{SCTM}), a non-inferiority test was performed. This non-inferiority test was applied for results from the occlusal site as a primary endpoint at a 1-sided significance level of 2.5% with an equivalence margin of δ =-1mm based on nonparametric tests. The null hypothesis for the non-inferiority is defined for the occlusal site: H_0: $\Delta_{VCMX} - \Delta_{SCTM} \leq -\delta$ and the alternative hypothesis: $\Delta_{VCMX} - \Delta_{SCTM} > -\delta$. If the non-inferiority can be shown, then in a hierarchical way, one may test for superiority.

The sample size calculation yielded 10 patients per group (total 20 patients) by taking into account a standard deviation of 0.5 mm per group, 90% power, and a drop-out rate of 30% without correction for the non-normality of the data.

RESULTS

Patient recruitment phase started in February 2012 and ended in February 2013. Twenty-one patients were screened and finally, a total of 20 patients entered the clinical trial having fulfilled all inclusion criteria. Ten patients (mean age 43.8 ± 13.2 years) were allocated to group VCMX (7 female, 3 male) and 10 patients (mean age 42.7 ± 19.1 years) to group SCTG (6 female, 4 male). A detailed description on patient demographics and augmentation sites is displayed in Tables 1A and 1B. All 20 included patients were operated and subsequently completed the study.

No relevant differences regarding baseline periodontal parameters were observed between the groups.

The median surgery time for the two groups amounted to 39.0min (Q1:33.0;Q3:51.0) (VCMX) and 34.0min (Q1:27.0;Q3:45) (SCTG) (p=0.319).

The clinicians considered the treatment to be successful in 9 out of 10 patients in both groups. In two patients (one in each group), clinically, no or only limited volume gain was observed and the therapies therefore considered being unsuccessful.

Soft tissue thickness

The median thickness of the mucosa at baseline was 3.5mm (2.5;4.9) (VCMX) and 3.8mm (3.0;5.0) (SCTG) (p=0.442) at the occlusal site, buccally, the respective values were 3.0mm (1.5;3.0) (VCMX) and 4.0mm (3.5;4.5) (SCTG) (p=0.211), and apically 2.0mm (1.0;3.0) (VCMX) and 3.0mm (2.5;3.5) (SCTG) (p=0.246). All data are given in Table 2A.

The changes between BL and FU-30 revealed a median increase in mucosal thickness of 1.0mm (0.0;2.0) (VCMX) (p=0.090) and 0.5mm (0.0;2.0) (SCTG) (p=0.156) at the occlusal site, of 1.0mm (1.0;3.0) (VCMX) (p=0.016) and 1.5mm (0.5;2.5) (SCTG) (p=0.086) buccally and by 2.5mm (1.0;4.0) (VCMX) (p=0.004) and 2.0mm (1.0;3.0) (SCTG) (p=0.141) apically. No statistically significant differences were found between VCMX and SCTG (p=0.987; p=0.953; p=0.481) for the changes between BL and FU-30.

The median thickness values at FU-30 [FU-90] were 4.0m (3.0;5.0) (VCMX) and 5.0mm (4.5;5.5); SCTG) (p=0.109) [4.25mm (3.5;6.0) (VCMX) and 4.0mm (4.0;6.5) (SCTG), p=0.613] at the occlusal site, buccally, the respective values were 4.5mm (4.0;5.0) (VCMX) and 5.8mm (4.5;7.0) (SCTG) (p=0.061) [4.0mm (3.5;4.5) (VCMX) and 5.3mm (4.5;5.5) (SCTG) p=0.136], and apically 4.8mm (4.0;5.0) (VCMX) and 5.0mm (3.5;7.0) (SCTG) (p=0.924) [2.5mm (2.0;4.5) (VCMX) and 5.0mm (4.0;7.0) (SCTG) p=0.081].

In terms of primary endpoint, the overall median soft tissue thickness increased between BL and FU-90 by 1.8mm (0.5;2.0) (VCMX) (p=0.018) and 0.5mm (-1.0;2.0) SCTG) (p=0.395) at the occlusal site. Hereby, the difference between the treatment related gains (primary objective) was 0.6 mm (difference of the medians 1.25); non-inferiority could be demonstrated (p=0.020). Superiority could not be shown. The two-sided nonparametric 95% confidence interval of the differences of the primary endpoint between the two groups was (-0.5, 2.0).

For all other sites, similar median increases were observed: 1.0mm (0.5;2.0) (VCMX) (p=0.074) and 1.5mm (-2.0;2.0) (SCTG) (p=0.563) buccally and 0.0mm (-0.5;1.5) (VCMX) (p=0.281) and 1.8mm (-0.5;3.3) (SCTG) (p=0.148) apically, respectively. No statistically significant differences were found between VCMX and SCTG (p=1.000; p=0.470).

The median changes between FU-30 and FU-90 amounted to 0.3mm (-0.5;0.5) (VCMX) (p=0.730) and -0.5mm (-10;1.0) (SCTG) (p=0.803) at the occlusal site, to -0.5mm (-0.5;0.5) (VCMX) (p=0.492) and -0.3mm (-1.0;0.8) (SCTG) (p=0.750) buccally and, to -1.5mm (-3.0;0.0) (VCMX) (p=0.016) and 1.0mm (0.0;2.0) (SCTG) (p=0.398) apically. Statistically significant differences were found between VCMX and SCTG apically (p=0.015), but not at the occlusal (p=0.513) and buccal site (p=0.914).

All data are displayed in Tables 2A and 2B.

Periodontal outcome measures

These measurements included KM, PPD, CAL, BOP, PlI values. For the width of keratinized tissue, no significant differences were observed between the two groups at the mesial tooth (p=0.264) and the target site (p=0.624). However, at the distal neighboring tooth, the difference in KM was statistically significantly between VCMX and SCTG (-1.1mm; Q1:-2.0; Q3:0.1) (p=0.029). All other outcome measures did only show minimal differences between the two groups (data not shown).

Patient-reported outcome measures (PROMs)

VAS scores were calculated four hours after the surgery and then daily until suture removal, as well at FU-30 and FU-90. Figure 4A demonstrates a slightly higher VAS score for SCTG between day 1 and day 3 post surgery without being statistically significantly different at any time-point (p>0.05). This correlated with a slightly higher consumption of analgesics from the day of surgery until suture removal for SCTG (5 tablets; Q1:2;Q3:7) compared to VCMX (3 tablets; Q1:1;Q3:6) (Figure 4B). Similar observations were made analyzing the OHIP-G14 questionnaire. Tables 3A and 3B show descriptive statistics for group differences for OHIP-14 subscores and total scores at baseline (BL), SR and FU-90 as well as for changes between SR and BL and between FU-90 and SR. In addition, p-values resulting from Mann-Whitney-U-tests (absolute values) and Wilcoxon signed rank test (comparing changes) of between-treatment and within-treatment differences were derived. All p values are above 0.10 and are therefore not reported in the tables. At suture removal, median overall scores for SCTG (5.0; Q1:3.0; Q3:11.0) were higher than for VCMX (3.0; Q1:0; Q3:6)) without reaching statistically significant differences (p=0.340). Median physical pain was 100% higher in group SCTG (3.0; Q1:1.0; Q3:3.0)) compared to group VCMX (1.5; Q1:0.0; Q3:2.0)) (p=0.113) at SR, thereby demonstrating a trend for VCMX to be associated with less morbidity. This was further underlined when analyzing the changes between baseline and SR. The

greatest differences between the groups (for changes between SR and BL) were found for physical pain (p=0.126) and social disability (p=0.187).

Safety evaluations

The status of the wound closure at the target site was assessed at day 7, 30 and 90 days. At the day of suture removal, 67% in group VCMX and 90% in group SCTG demonstrated a complete wound closure. This difference was not statistically significant and demonstrated an ODDs ratio of 0.22 (95% confidence interval 0.018-2.674). In 5/10 patients (VCMX) and 2/20 patients (SCTG) respectively, swelling was present at the same visit. At the two later follow-up time-points, all target sites were completely closed and no swelling was observed. No serious AE and no device-related AE were observed during the entire study period. The total number of AEs in the study was 20 (VCMX: 7; SCTG: 13). This predominantly encompassed gastrointestinal disorders, general infections and dizziness.

Descriptive histology and histomorphometry

Eighteen biopsies (VCMX=10; SCTG=8) could be harvested and 17 could be further processed for histologic analyses. Biopsies in group SCTG revealed a relatively loose network of collagen fibers with few inflammatory cells. No differentiation between transplanted connective tissue (CT) and newly formed CT was possible. In some specimens, bulks of adipocytes and few glandular cells were present. Vascularization was observed throughout the specimens with a relatively high number of smaller blood vessels (Figure 5A). In group VCMX, a dense collagen fiber network was present and the VCMX matrix could clearly be identified (Figure 5B). The matrix body, to some extent, revealed turn-over and remodeling processes. In some parts, the VCMX body was surrounded by a dense connective tissue, in other parts, by a looser network of newly formed collagen fibers. Thick elastic fibers were part of the VCMX body. Vascularization was present throughout the specimens. The number of inflammatory cells was limited. The histomorphometric assessment revealed a remaining matrix body of 32.1%

(±18.5%) and a mean amount of CT of 30.1% (±11.8%) (VCMX). In group SCTG, the mean amount of CT (transplanted and newly formed tissue) was 77.6% (±11.6%). The differences in CT was significant ($p<0.05$), but not the density of the harvested biopsies (background) ($p>0.05$).

DISCUSSION

The present RCT demonstrated that i) the soft tissue thickness increased up to 1.6mm with only minimal changes until the last follow-up without statistical differences between the groups; ii) soft tissue augmentation at implant sites was a safe procedure with a minimal number of complications using SCTG or VCMX; iii) PROMs, in general, favored the use of the VCMX and, iv) both, SCTG and VCMX integrated well into the surrounding soft tissues.

With respect to the primary objective, the non-inferiority of VCMX could not be shown, since the study was underpowered for that specific analysis. This was due to the fact that the variability assumed in the sample size calculation was too low. However, the sample size was appropriate for describing the outcome variables and the respective changes.

Various techniques and materials were suggested in the past to augment soft tissue volume prior to or simultaneous with implant placement, during the healing phase of the implant, at abutment connection or even after the insertion of the final reconstruction (Thoma et al., 2014b). Clinically, the timing and the decision on whether or not to augment depends on the clinician's choice, the patient's acceptance for the procedure and the clinical need (Levine et al., 2014). Even though a variety of materials including allogenic- and xenogenic materials were applied for that purpose, the use of the subepithelial connective tissue graft (SCTG) has been considered to be the gold standard (Thoma et al., 2014a). Clinical studies evaluating the increase in soft tissue volume following augmentation with SCTGs reported a range between 0.35-3.2mm depending on the location and follow-up time point (Thoma et al., 2014a, Eghbali et al., 2014, De Bruyckere et al., 2015). In the present study the increase in soft tissue volume was assessed at three levels (on top of the implant, slightly buccal and more apical) and ranged between 0.56-1.56mm. Research activities in more recent years focused on the development of alternatives to replace autogenous soft tissues. This was

predominantly driven by a number of limitations and disadvantages associated with the use of autogenous tissue. Among these drawbacks, the donor site appears to be the critical issue resulting in an increased morbidity and a reduced patient's acceptance for the procedure (Griffin et al., 2006, Zucchelli et al., 2010, Cairo et al., 2012, Harris et al., 2005, Del Pizzo et al., 2002). Only few clinical studies evaluated soft tissue substitutes for oral soft tissue volume augmentation. The obtained increase in soft tissue volume ranged between 0.35-2.14mm (Batista et al., 2001, Simion et al., 2012). The lack of longer-term data might in part be explained by a lack of performance of the tested soft tissue substitutes. The soft tissue substitute used in the present study has been successfully tested in a number of preclinical studies (Thoma et al., 2011, Thoma et al., 2010, Thoma et al., 2012b). Apart from favorable tissue integration, a soft tissue volume increase non-inferior to the SCTG was reported (Thoma et al., 2010). These results are supported by the present study. The obtained increase in soft tissue thickness was similar to the SCTG without statistically significant differences at any of the three measured sites.

From a clinician's and a patient's perspective, a number of other parameters may play an important role in the decision-making process for using an autogenous soft tissue graft or a soft tissue substitute. This mainly involves PROMs (McGrath et al., 2012, Lang et al., 2012). Two kinds of questionnaires were used in the present study to evaluate PROMs: OHIP-14 and VAS scores. The OHIP-14 questionnaire is a short-form of the OHIP-49 assessing associations with socio-ethnographic and clinical oral status variables. This questionnaire demonstrated good reliability, validity and precision without significant differences compared to the OHIP-49 for dentate patients (Slade, 1997). The applicability of OHIP-14 to evaluate soft tissue grafting procedures has not been evaluated, however, and – indeed - the right skewed data indicate limited sensitivity for low severity of complaints. In the present study, the VAS scores during the first three days after surgery were slightly higher for the SCTG group compared to the VCMX group and a higher consumption of analgesics for the SCTG group. This was underlined with OHIP-14 data demonstrating 50% more physical pain for SCTG compared to VCMX at SR

and the greatest differences in change (SR vs. BL) for physical pain (1.1, p=0.112) and social disability (0.9, p=0.123). These data are in line with other studies reporting on the use of soft tissue substitutes compared to autogenous soft tissue grafts (Sanz et al., 2009, Lorenzo et al., 2012). In these studies, more favorable PROMs were reported for soft tissue substitutes compared to the use of autogenous soft tissue grafts involving less overall pain and a reduced consumption of analgesics. In addition, the same studies reported less surgical time needed to perform the surgeries in the groups with soft tissue substitutes, explaining it with the lack of a second surgical site. In the present study, the surgical time needed to perform soft tissue volume augmentation was not statistically significantly different between the two groups. Even though, in the VCMX group, no second surgical site (donor site) was needed, a similar surgical time was calculated. This finding might be explain by the following reasons: i) the learning curve associated with a new device compared to a routinely performed procedure (SCTG), ii) the skills and experience of the surgeons and, iii) a negligible time needed for the harvesting procedure relative to the duration of the entire surgery. Only surgeons experienced in soft tissue grafting procedures performed the surgical interventions. The high level of clinical skills and the routine use of these interventions probably masked the effect and influence of the second surgical site on the surgical time.

No serious adverse events (AEs) and no device-related AEs were observed in the present study demonstrating that both materials can safely be used for the applied intervention. The total number of AEs however revealed a higher number associated with the group SCTG (13 compared to 20 in group VCMX). These AEs predominantly included general infections and gastrointestinal disorders, which might also be attributed to the concomitant medication such as antibiotics. Clinically, the status of the wound closure revealed a higher number of dehiscences present at the day of suture removal translated into an ODDs ratio of 0.22. This higher rate of clinical complications may be due to the above-mentioned learning curve with the new soft tissue substitute (Chambers, 2012). The VCMX used in the present study, has not been tested in the

clinic before and the surgical technique was adapted from the routine procedure with SCTGs. This technique may not be ideal for the use of this new device and might have to be adapted to the new device in the future. In contrast to SCTGs, the VCMX feature a high elasticity and are being soaked with blood immediately. Any pressure applied onto the device during surgery lead to an initial compression of the device, which within minutes reached the initial volume again. This elasticity is clearly in contrast to SCTGs (being relatively resistant to compression) and requires a completely tension-free wound closure. Even though, 33% of VCMX sites demonstrated a dehiscence at 7 days, the further healing was free of complications and complete wound closure was observed in all sites at 30 days. A complication-free healing following wound dehiscences and no signs of foreign body reactions with the applied cross-linked VCMX is in contrast to previous preclinical and clinical studies that demonstrated a high rate of wound dehiscences and subsequent infections using cross-linked devices (Becker et al., 2009, Annen et al., 2011).

Histologic data provide a reliable instrument to analyze tissue integration and to assess the turn-over of implanted autogenous tissue and soft tissue substitutes (Ghanaati et al., 2011). The integration of autogenous tissue and VCMX into surrounding hard and soft tissues was evaluated in a recent preclinical study. The study reported a distinct border between the SCTG and the underlying bone and a tight integration towards the covering buccal flap without a clear distinction between augmented tissue and covering flap, whereas the bulk of the SCTG consisted of a dense connective tissue, adipocytes, glandular cells and blood vessels at 3 months. VCMX sites revealed an encapsulated and well-organized collagen matrix network infiltrated with newly formed connective tissue at 1 month. Two months later, tissue integration of VCMX had increased and remodeling processes and turn-over had taken place (Thoma et al., 2011). These data correlate with the findings of the present study. SCTG biopsies revealed a relatively loose connective tissue network reaching 78% of the volume, whereas VCMX sites consisted of almost identical amounts of remaining collagen matrix (32%) and newly formed

connective tissue (30%) with only few inflammatory cells present. This demonstrated that the newly developed VCMX integrated well into the surrounding soft tissues allowing remodeling processes and enhancing the formation of new connective tissue within the matrix body in way consistent with data from previous preclinical studies (Thoma et al., 2011, Thoma et al., 2012b).

The present study was designed as a RCT to demonstrate non-inferiority. A power calculation was performed and based on the only data for VCMX that were available at the time of the protocol development (Thoma et al., 2011). Since no clinical data were available at that time, using a similar clinical protocol, interindividual patient differences and variations with respect to measured outcomes were underestimated. This resulted in a study design underpowered to demonstrate a non-inferiority model. Future studies can be designed using a power calculation based on the data of the present study These clinical trials will be needed to further assess soft tissue volume augmentation surgeries at implant sites and to demonstrate that VCMX can replace autogenous tissue for this type of intervention. Moreover, such clinical studies should focus on a longer-term follow-up similar to what has been reported for other soft tissue substitutes for root coverage procedures (Harris, 2004).

CONCLUSIONS

The use of the three-dimensionally stable collagen matrix and the subepithelial connective tissue graft for soft tissue augmentation at implant sites rendered a similar gain in soft tissue volume. Only minimal changes in soft tissue thickness were observed between the first and last follow-up at 90 days. The surgical procedures using VCMX or SCTG were safe with only a minimal number of complications. Patient-reported outcome measures, slightly favored the use of VCMX without significant differences. The histological analysis revealed well-integrated grafts without foreign body reactions in both groups. The VCMX allowed remodeling processes and enhanced the formation of new connective tissue within the matrix body. Both, VCMX and SCTG can be effectively and safely used for the soft tissue augmentation at implant sites resulting in an increase in soft tissue volume up to 90 days based on this RCT. Clinical studies with a longer-term follow-up, however, will be needed in the future to support these early short-term based findings.

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FIGURE LEGENDS

Figure 1: Flow-chart depicting visits, time-line and evaluated parameters. PROMs=patient-reported outcome measures. FU-30=follow-up at 30 days. FU-90=follow-up at 90 days.

Figure 2. Clinical procedures represented with one case (Collagen matrix). A. Clinical situation at screening. B. Prepared flap on top of ridge (full thickness) and on buccal side (split-thickness). C. Collagen matrix placed on top of the ridge and underneath the buccal flap. D. Primary wound closure. E. Suture removal at 7-10 days. F. Follow-up examination at 30 days (FU-30). G. Follow-up examination at 90 days (FU-90), roll flap to access dental implant. H. Healing abutment placed. I. Final reconstruction in situ (not part of the present study).

Figure 3A: Virtual 3-D model of the individualized stent.

Figure 3B: Individualized stent for transmucosal probing in situ.

Figure 4A: Mean VAS scores for pain perceived by patients at different time-points including standard deviations.

Figure 4B: Mean amount of analgesics (500mg tablets of mefenacid) consumption per day including standard deviations.

Figure 5: Histological section (Van Gieson-Elastica, x500 magnification). A. SCTG=subepithelial connective tissue graft; B. VCMX= collagen matrix. VCMX=collagen matrix body. CT=connective tissue. EF=elastic fiber. BV=blood vessel.

Table 1A: Patient demographics and p-values (Mann-Whintey test)SD=standard deviation. Q1=first quartile. Q3=third quartile. VCMX=collagen matrix. SCTG=subepithelial connective tissue grafts.

Table 1B: Location and number of augmented sites for each group. VCMX=collagen matrix. SCTG=subepithelial connective tissue grafts.

Table 2A: Mean baseline soft tissue thickness and p-values (Wilcoxon signed rank and Mann-Whitney test). SD=standard deviation. Q1=first quartile. Q3=third quartile. VCMX=collagen matrix. SCTG=subepithelial connective tissue grafts.

Table 2B: Change in soft tissue thickness and p-values (Wilcoxon signed rank and Mann-Whitney test). SD=standard deviation. Q1=first quartile. Q3=third quartile. VCMX=collagen matrix. SCTG=subepithelial connective tissue grafts. FU-30=Follow-up at 30 days. FU-90=Follow-up at 90 days.

Table 3A: OHIP-14 domains and total scores: Descriptive statistics (number of cases (N), mean, standard deviation (SD), median, first (Q1) and third quartile (Q3)) of absolute values at baseline (top), sutural removal (=day 7) and at day 90

Table 3B: OHIP-14 domains and total score: Descriptive statistics (number of cases (N), mean, standard deviation (SD), median, first (Q1) and third quartile (Q3)) changes at sutural removal (=day 7) vs. baseline (top) and changes at day 90 vs. sutural removal (bottom).

Table 1A

		VCMX (n=10)	SCTG (n=10)	p-value
Gender	n (female)	7	6	1.000
	n (male)	3	4	
Age	Mean	43.8	42.7	1.000
	SD	13.2	19.1	
	Median	45.0	46.7	
	Q1 ; Q3	39.1 ; 47.8	22.4 ; 60.1	
	p-value	0.002	0.002	
Cigarettes per day	Mean	0.8	1.0	1.000
	SD	2.5	2.5	
	Median	0.0	0.0	
	Q1 ; Q3	0.0 ; 0.0	0.0 ; 0.0	
	p-value	1.000	0.500	

Table 1B

Site	15	14	13	12	11	21	22	23	24	25
VCMX	1				3	2			1	3
SCTG				2	2	4	1			

Site	45	44	43	42	41	31	32	33	34	35
VCMX										
SCTG						1				

Table 2A

		VCMX [mm]	SCTG [mm]	p-value
Mucosal thickness of occlusal [mm]	n Mean ± SD Median Q1 ; Q3 p-value	10 3.4 ± 1.0 3.5 2.5 ; 4.0 0.002	10 4.2 ± 1.9 3.8 3.0 ; 5.0 0.002	0.442
Mucosal thickness of buccal [mm]	n Mean ± SD Median Q1 ; Q3 p-value	9 2.9 ± 1.5 3.0 1.5 ; 4.0 0.004	9 4.1 ± 2.0 4.0 3.5 ; 4.5 0.004	0.211
Mucosal thickness of apical [mm]	n Mean ± SD Median Q1 ; Q3 p-value	10 2.6 ± 2.3 2.0 1.0 ; 3.0 0.002	9 3.4 ± 1.8 3.0 2.5 ; 3.5 0.004	0.246

Table 2B

		VCMX [mm]				SCTG [mm]				
		n	Mean ± SD	Median (Q1 ; Q3)	p-value within group	n	Mean ± SD	Median (Q1 ; Q3)	p-value within group	p-value between group
Baseline to FU-30	occlusal	10	0.9 ± 1.4	1.0 (0.0 ; 2.0)	0.090	10	0.9 ± 1.5	0.5 (0.0 ; 2.0)	0.156	0.987
	buccal	9	1.7 ± 1.6	1.0 (1.0 ; 3.0)	0.016	9	1.6 ± 2.3	1.5 (0.5 ; 2.5)	0.086	0.953
	apical	10	2.5 ± 1.6	2.5 (1.0 ; 4.0)	0.004	9	1.5 ± 2.6	2.0 (1.0 ; 3.0)	0.141	0.481
Baseline to FU-90	occlusal	10	1.4 ± 1.4	1.8 (0.5 ; 2.0)	0.018	10	0.8 ± 1.8	0.5 (-1.0 ; 2.0)	0.395	0.359
	buccal	9	1.1 ± 1.4	1.0 (0.5 ; 2.0)	0.074	7	0.8 ± 2.2	1.5 (-2.0 ; 2.0)	0.563	1.000
	apical	10	0.9 ± 1.9	0.0 (-0.5 ; 1.5)	0.281	8	1.6 ± 2.6	1.8 (-0.5 ; 3.3)	0.148	0.470
FU-30 to FU-90	occlusal	10	0.5 ± 1.9	0.3 (-0.5 ; 0.5)	0.730	10	-0.1 ± 1.4	-0.5 (-1.0 ; 1.0)	0.803	0.513
	buccal	10	-0.4 ± 1.4	-0.5 (-0.5 , 0.5)	0.492	8	-0.4 ± 2.0	-0.3 (-1.0 ; 0.8)	0.750	0.914
	apical	10	-1.7 ± 1.7	-1.5 (-3.0 ; 0.0)	0.016	9	0.6 ± 2.0	1.0 (0.0 ; 2.0)	0.398	0.015

Table 3A

	OHIP domains and total score: absolute values at baseline					
	VCMX			SCTG		
	N	Mean ± SD	Median (Q1 ; Q3)	N	Mean ± SD	Median (Q1 ; Q3)
Functional limitation	10	0.6 ± 1.3	0 (0 ; 1)	10	0.8 ± 0.8	1 (0 ; 1)
Physical pain	10	1.0 ± 1.6	0 (0 ; 1)	10	1.0 ± 1.1	1 (0 ; 2)
Psychol. discomfort	10	1.2 ± 2.1	0 (0 ; 1)	10	0.8 ± 1.3	0 (0 ; 1)
Physical disability	10	0.3 ± 0.7	0 (0 ; 0)	10	0.7 ± 1.2	0 (0 ; 2)
Physiol. disability	10	1.2 ± 2.0	0 (0 ; 2)	10	1.1 ± 1.3	0.5 (0 ; 2)
Social disability	10	0.8 ± 1.6	0 (0 ; 1)	10	0.2 ± 0.6	0 (0 ; 0)
Handicap	10	0.5 ± 1.3	0 (0 ; 0)	10	0.6 ± 1.0	0 (0 ; 1)
Overall	10	5.6 ± 9.5	2 (1 ; 4)	10	5.2 ± 6.1	3 (1 ; 9)
	OHIP domains and total score: absolute values at SR					
	VCMX			SCTG		
	N	Mean ± SD	Median (Q1 ; Q3)	N	Mean ± SD	Median (Q1 ; Q3)
Functional limitation	10	0.9 ± 1.4	0 (0 ; 1)	10	1.0 ± 1.2	1 (0 ; 1)
Physical pain	10	1.5 ± 1.7	1.5 (0 ; 2)	10	2.6 ± 1.6	3 (1 ; 3)
Psychol. discomfort	10	1.2 ± 1.7	0.5 (0 ; 2)	10	1.1 ± 1.5	0.5 (0 ; 2)
Physical disability	10	0.5 ± 1.0	0 (0 ; 1)	10	0.5 ± 0.7	0 (0 ; 1)
Physiol. disability	10	1.0 ± 1.5	0 (0 ; 2)	10	0.9 ± 1.1	0.5 (0 ; 2)
Social disability	10	0.6 ± 1.3	0 (0 ; 0)	10	0.9 ± 1.9	0 (0 ; 1)
Handicap	10	0.4 ± 1.0	0 (0 ; 0)	10	0.6 ± 1.1	0 (0 ; 1)
Overall	10	6.1 ± 8.8	3 (0 ; 6)	10	7.6 ± 6.7	5 (3 ; 11)
	OHIP domains and total score: absolute values at day 90					
	VCMX			SCTG		
	N	Mean ± SD	Median (Q1 ; Q3)	N	Mean ±SD	Median (Q1 ; Q3)
Functional limitation	10	0.9 ± 1.3	0 (0 ; 2)	9	0.4 ± 0.9	0 (0 ; 0)
Physical pain	10	0.7 ± 0.9	0 (0 ; 2)	8	1.1 ± 1.2	1 (0 ; 2)
Psychol. discomfort	10	1.1± 1.3	0.5 (0 ; 2)	9	1.0 ± 1.1	1 (0 ; 2)
Physical disability	10	0.3 ± 0.9	0 (0 ; 0)	9	0.7 ± 1.1	0 (0 ; 1)
Physiol. disability	10	0.9 ± 1.4	0 (0 ; 2)	9	0.9 ± 1.2	0 (0 ; 2)
Social disability	10	0.4 ± 1.0	0 (0 ; 0)	9	0.2 ± 0.7	0 (0 ; 0)

	OHIP domains and total score: absolute values at baseline					
	VCMX			SCTG		
	N	Mean ± SD	Median (Q1 ; Q3)	N	Mean ± SD	Median (Q1 ; Q3)
Handicap	10	0.3 ± 0.7	0 (0 ; 0)	9	0.7 ± 1.0	0 (0 ; 1)
Overall	10	4.6 ± 5.9	1.5 (0 ; 8)	10	4.4 ± 5.6	3 (0 ; 7)

Table 3B

OHIP domains and total score: Change at SR vs. baseline						
	VCMX			SCTG		
	N	Mean (±SD)	Median (Q1 ; Q3)	N	Mean (±SD)	Median (Q1 ; Q3)
Functional limitation	10	0.3 ± 1.1	0 (0 ; 0)	10	0.2 ± 1.1	0 (-1 ; 1)
Physical pain	10	0.5 ± 1.4	0.5 (0 ; 2)	10	1.6 ± 1.6	2 (0 ; 3)
Psychol. discomfort	10	0.0 ± 1.1	0 (-1 ; 1)	10	0.3 ± 0.8	0 (0 ; 1)
Physical disability	10	0.2 ± 0.4	0 (0 ; 0)	10	-0.2 ± 1.2	0 (-1 ; 0)
Physiol. disability	10	-0.2 ± 1.4	0 (-1 ; 0)	10	-0.2 ± 1.2	0 (-1 ; 0)
Social disability	10	-0.2 ± 1.1	0 (0 ; 0)	10	0.7 ± 1.3	0 (0 ; 1)
Handicap	10	-0.1 ± 0.3	0 (0 ; 0)	10	0.0 ± 0.5	0 (0 ; 0)
Overall	10	0.5 ± 3.1	0.5 (-1 ; 3)	10	2.4 ± 5.0	2 (0 ; 4)
OHIP domains and total score: Change at day 90 vs. SR						
	VCMX			SCTG		
	N	Mean (±SD)	Median (Q1 ; Q3)	N	Mean (±SD)	Median (Q1 ; Q3)
Functional limitation	10	0.0 ± 0.8	0 (0 ; 0)	9	-0.6 ± 1.1	0 (-1 ; 0)
Physical pain	10	-0.8 ± 1.1	0 (-2 ; 0)	8	-1.6 ± 2.0	-1.5 (-3 ; 0)
Psychol. discomfort	10	-0.1 ± 1.3	0 (-1 ; 1)	9	-0.2 ± 0.8	0 (0 ; 0)
Physical disability	10	-0.2 ± 1.2	0 (0 ; 0)	9	0.1 ± 1.2	0 (0 ; 1)
Physiol. disability	10	-0.1 ± 0.9	0 (0 ; 0)	9	-0.1 ± 0.8	0 (0 ; 0)
Social disability	10	-0.2 ± 1.0	0 (0 ; 0)	9	-0.8 ± 1.4	0 (-1 ; 0)
Handicap	10	-0.1 ± 0.6	0 (0 ; 0)	9	0.0 ± 0.7	0 (0 ; 0)
Overall	10	-0.6 ± 4.1	0 (-2 ; 2)	10	-1.3 ± 2.5	-1.5 (-3 ; 0)

Figure 1

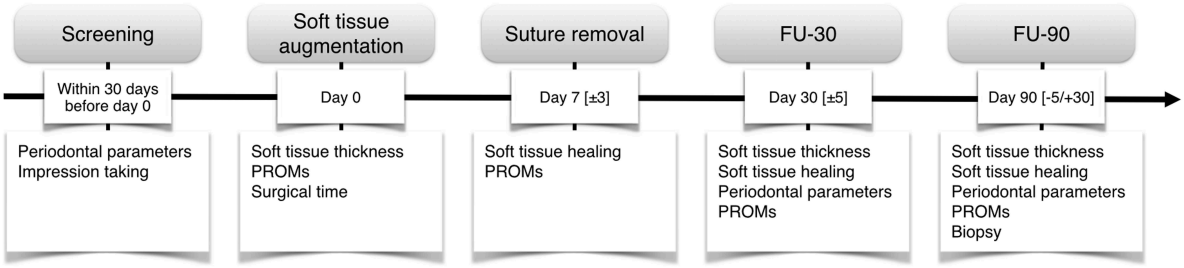
Screening
Soft tissue augmentation
Suture removal
FU-30
FU-90
Within 30 days before day 0
Day 0
Day 7 [±3]
Day 30 [±5]
Day 90 [-5/+30]
Periodontal parameters
Impression taking
Soft tissue thickness
PROMs
Surgical time
Soft tissue healing
PROMs
Soft tissue thickness
Soft tissue healing
Periodontal parameters
PROMs
Soft tissue thickness
Soft tissue healing
Periodontal parameters
PROMs
Biopsy

Figure 2A

Figure 2B

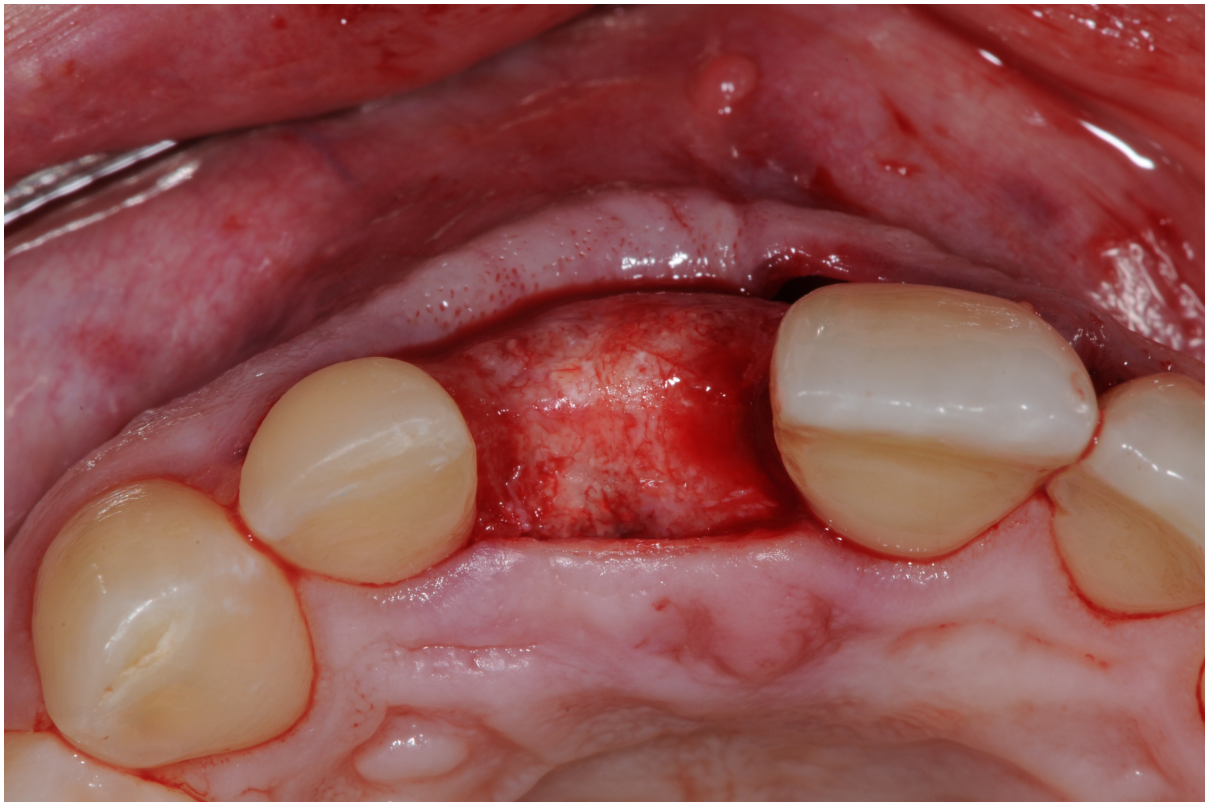

Figure 2C

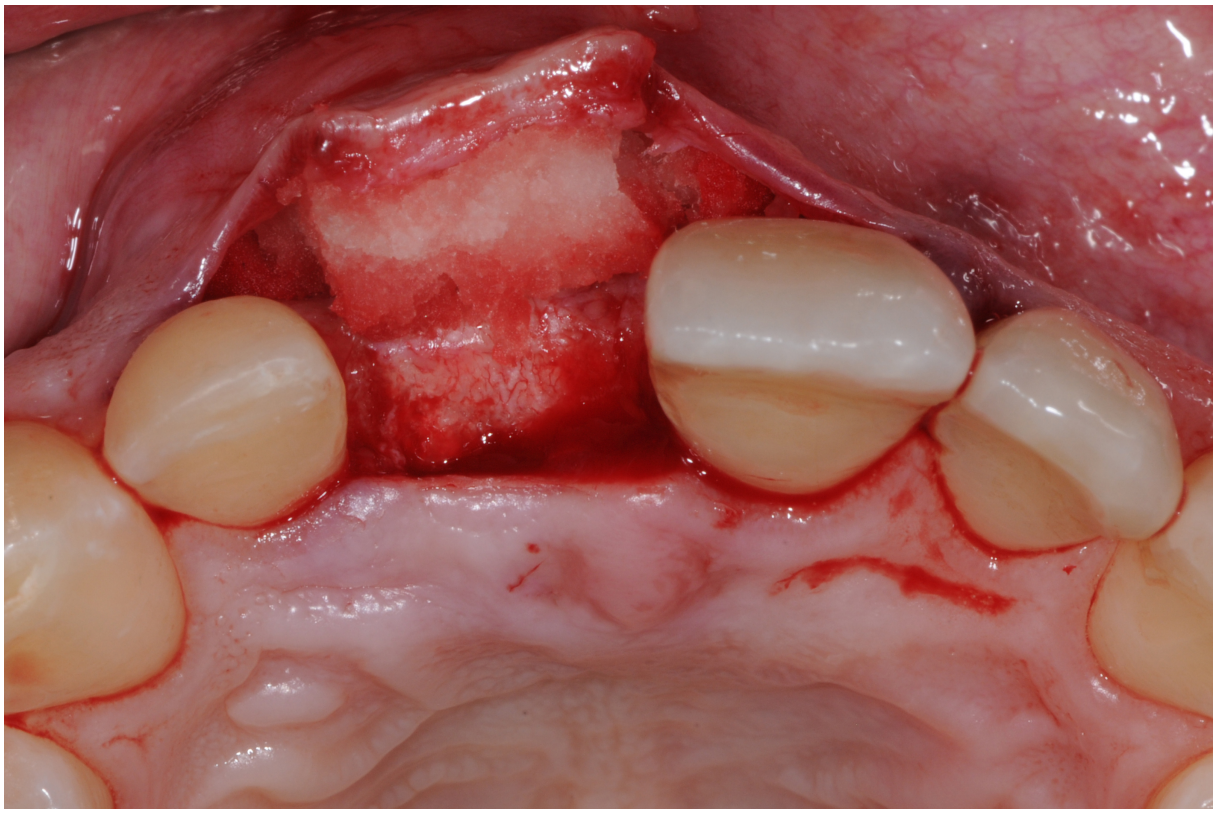

Figure 2D

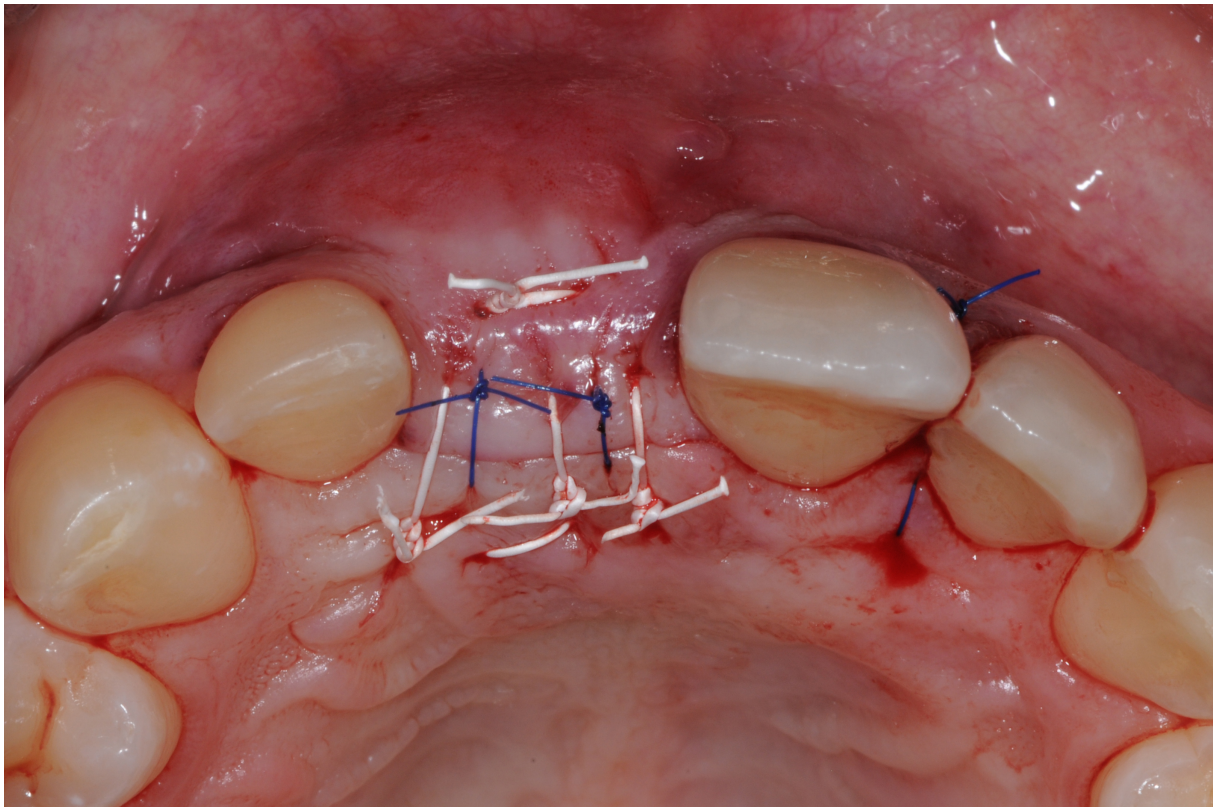

Figure 2E

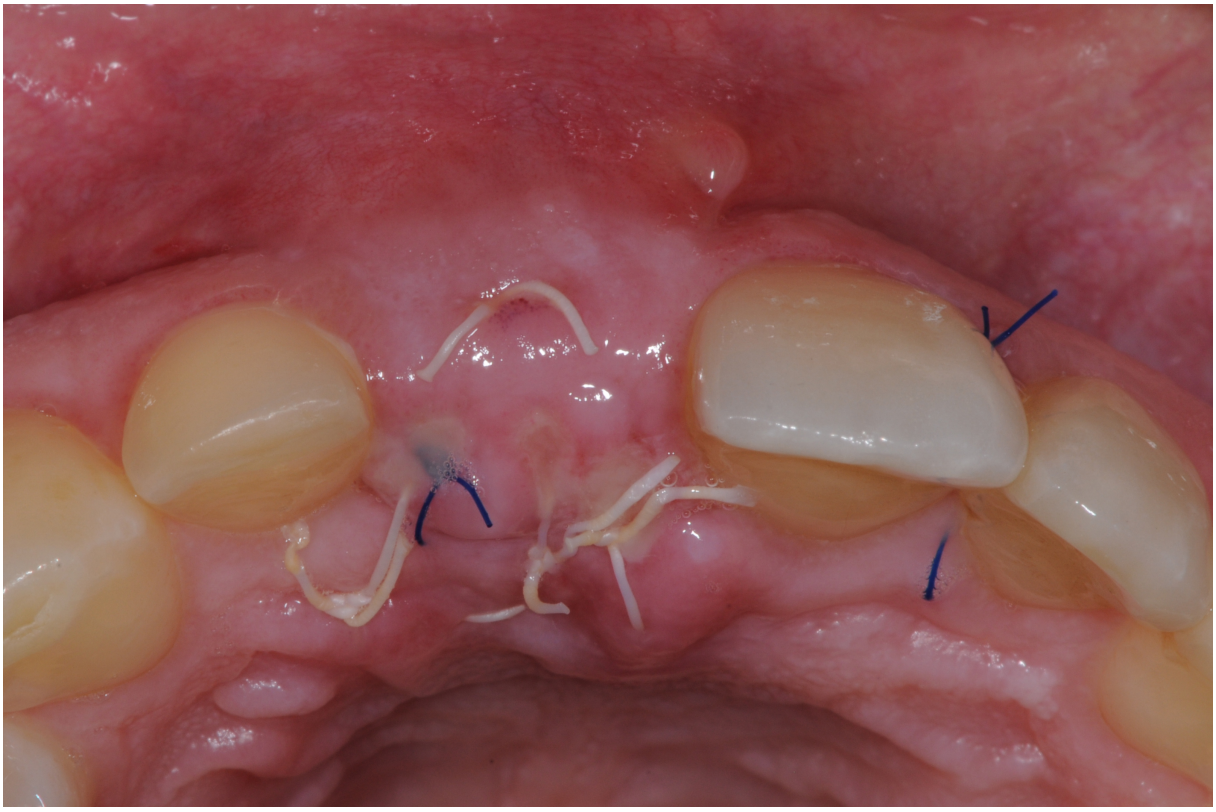

Figure 2F

Figure 2G

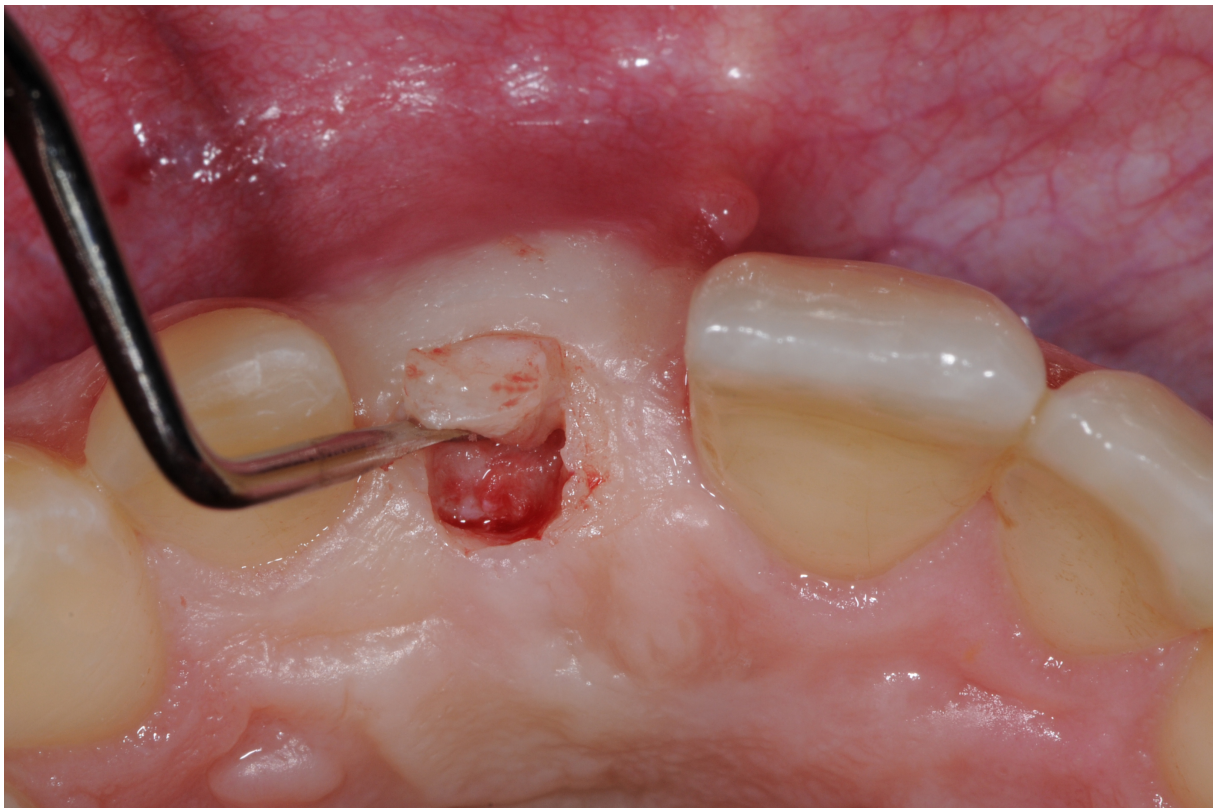

Figure 2H

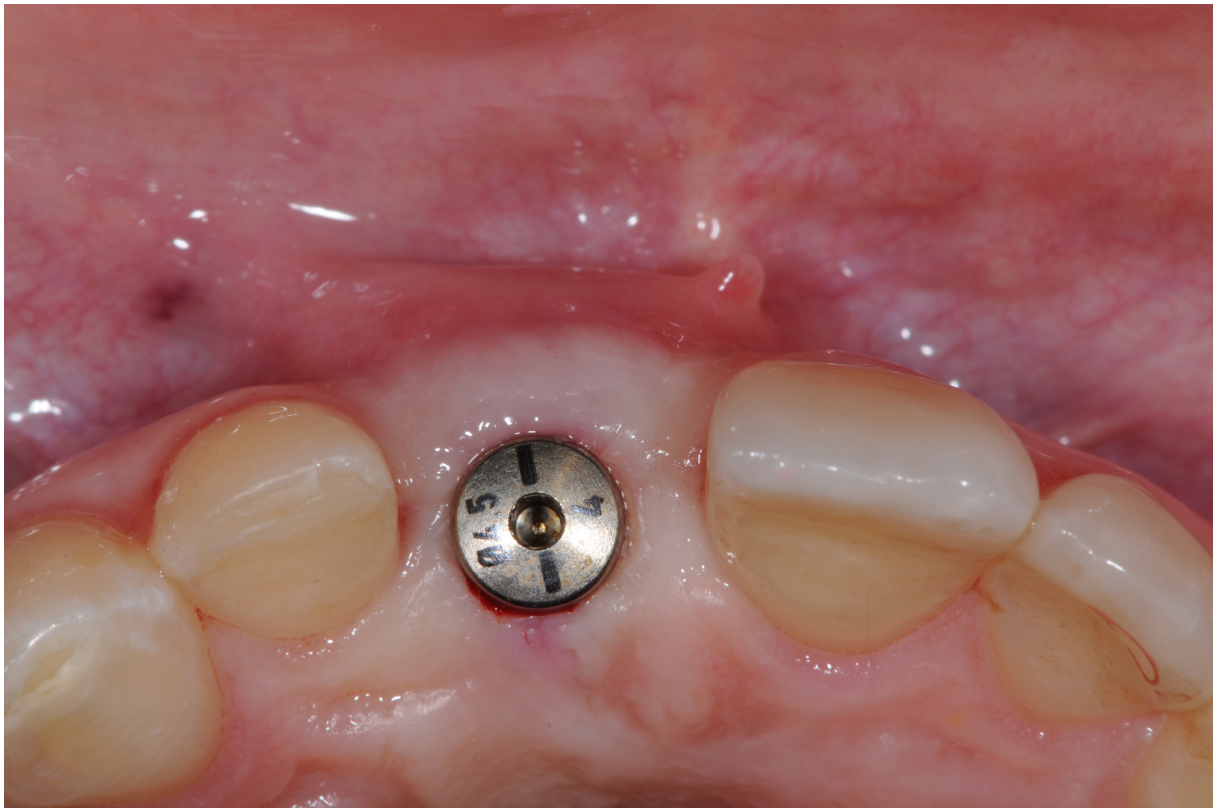

Figure 2I

Figure 3A

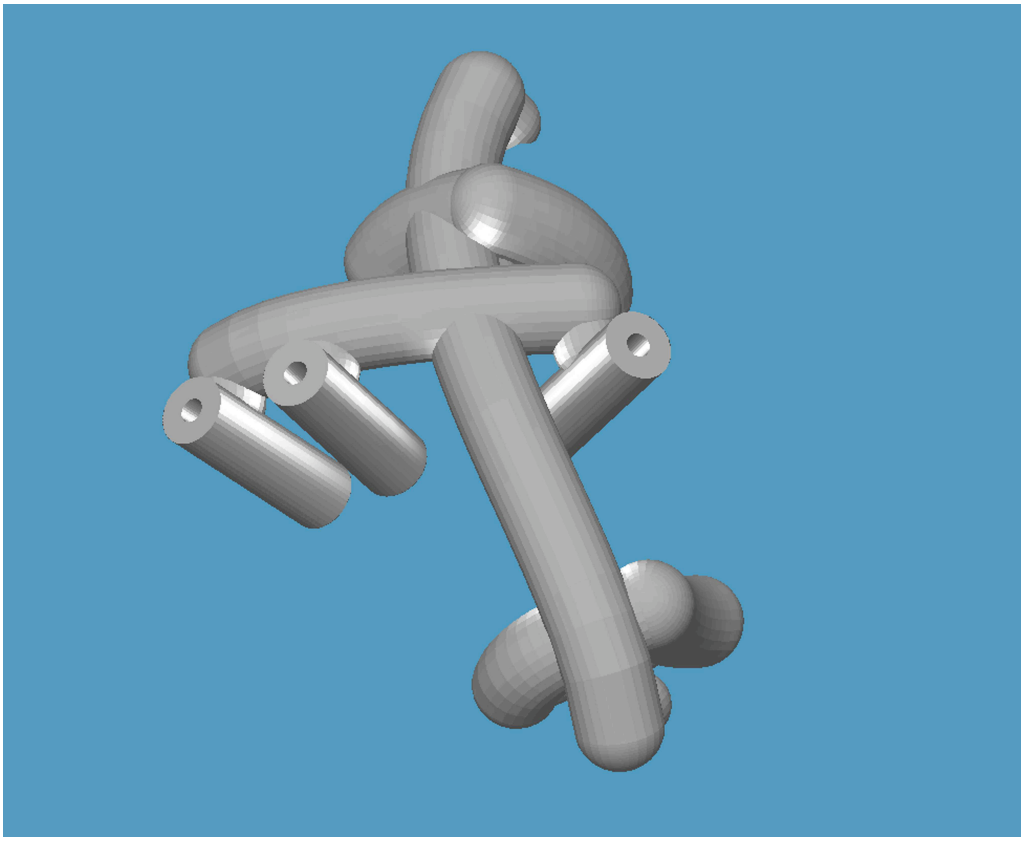

Figure 3B

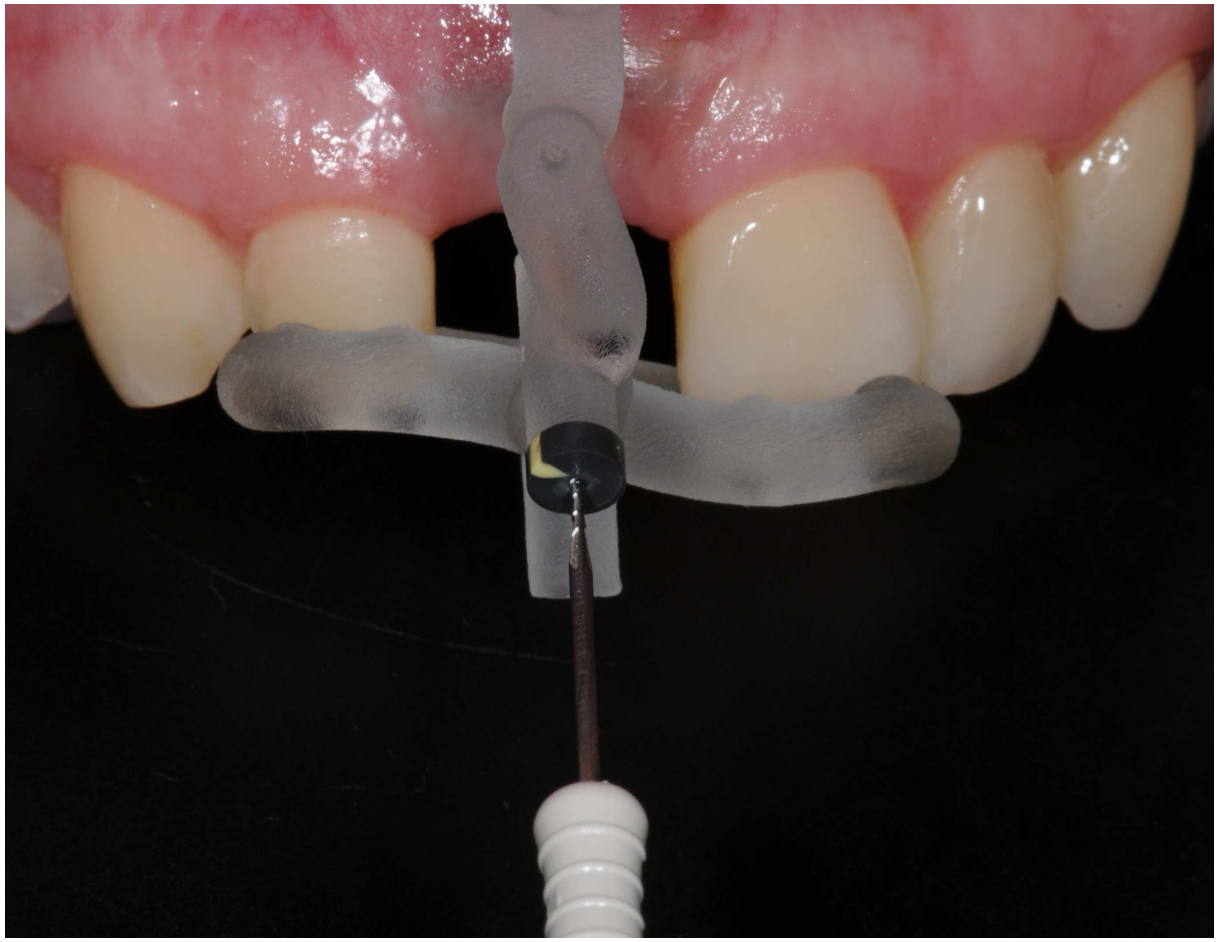

Figure 4A

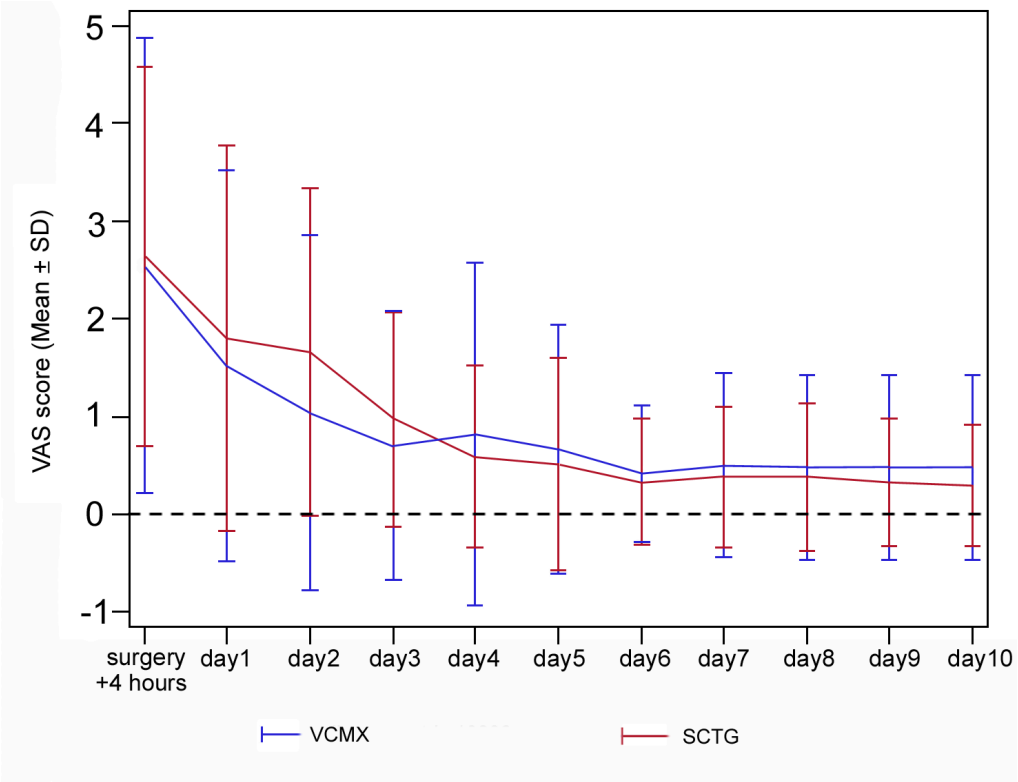

Figure 4B

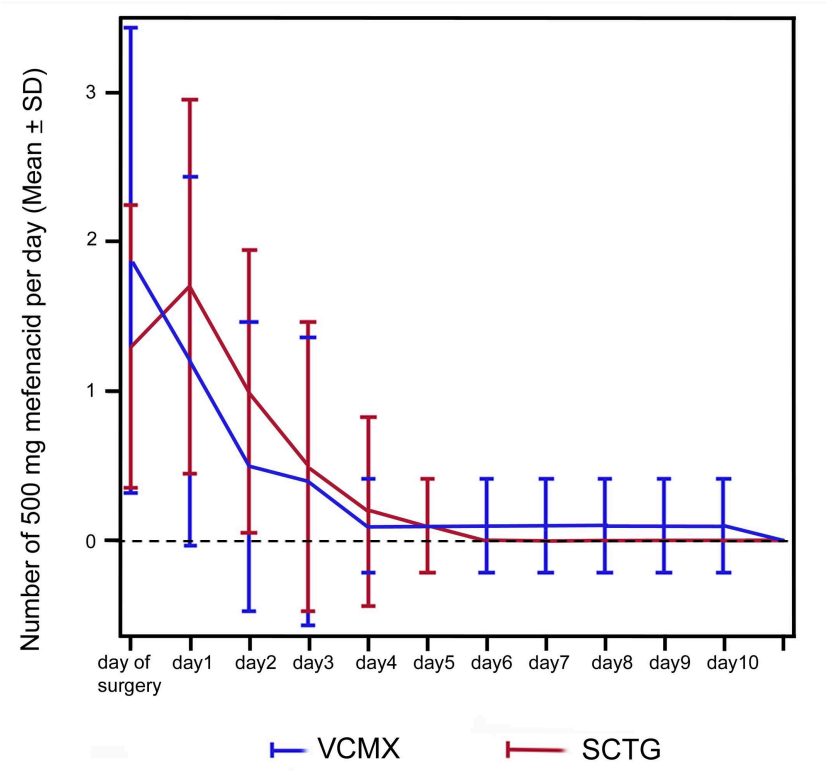

Figure 5A

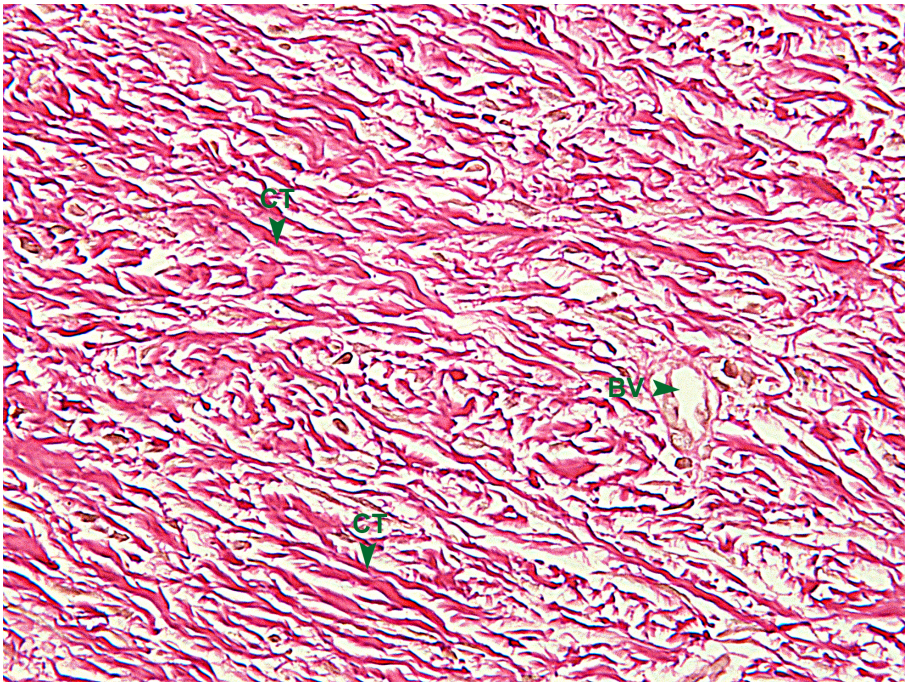

Figure 5B